

NORTHERN ILLINOIS UNIVERSITY

Characterization of The Binding Thermodynamics of Metal

Cofactors to *Burkholderia pseudomallei* IspF

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Requirements of the Baccalaureate Degree

With Upper Division Honors

Department of Chemistry and Biochemistry

By

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DeKalb, Illinois

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**HONORS THESIS ABSTRACT
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BIBLIOGRAPHY:

¹Odom, A.R; Five Questions about Non-Mevalonate Isoprenoid Biosynthesis. *PLoS Pathog.* **2011**. 7(12). St. Louis. Washington University School of Medicine. <https://doi.org/10.1371/journal.ppat.1002323>

²Buhaescu, I; Izzedine, H; Mevalonate pathway: a review of clinical and therapeutical implications. *ClinBiochem.* **2007**. 40.575–584.

³Lichtenthaler, H; The 1-Deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol.* **1999**. 50.47-65. doi:10.1146/annurev.arplant.50.1.47. PMID 15012203

⁴Begley, D. et al; Leveraging structure determination with fragment screening for infectious disease drug targets: MECP synthase from *Burkholderia pseudomallei*.*Springerlink.com*.**2011**

⁵Centers for Disease Control and Prevention. Melioidosis. (2012). <https://www.cdc.gov/melioidosis/>

⁶Mayaux Ayaux, J.-F.;Kalogerakos, T.; Brito, K. K; and Blanquet, S; Removal of the Tightly Bound Zinc from *Escherichia coli* Trypsin-Modified Methionyl-tRNA Synthetase. *European Journal of Biochemistry.* **1982**.128. 41–46. doi:10.1111/j.1432-1033.1982.tb06928.x

⁷Lauw, S.; Characterization and inhibition of the IspC, IspE and IspF proteins involved in the deoxyxylulose phosphate pathway of isoprenoids biosynthesis. **2008**.

⁸Milev,S.;Isothermal titration calorimetry: Principles and experimental design. *General Electric*.**2003**

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ABSTRACT (100-200 WORDS): The characterization of the binding properties of the Zinc ion, a metal cofactor, for the IspF enzyme can reveal details of critical interactions that allows to better understand IspF function. The use of Isothermal Titration Calorimetry (ITC) was used to determine the binding affinity of the Zn^{2+} cofactor to the IspF enzyme. A sample of IspF was titrated with a Zinc solution, using a Zinc to Buffer titration as a control. This was done to isolate ligand and protein interactions from the interference of buffer solution. The titration of the Zn^{2+} cofactor can reveal insight into the binding thermodynamics with *Burkholderia pseudomallei* IspF. Further studies will be carried out against another IspF sample from *Escherichia coli*, as well as other 2+ metal ions like Magnesium (Mg^{2+}) and Calcium (Ca^{2+}).

University Honors Program

Capstone Approval Page

Capstone Title (print or type)

Characterization of The Binding Thermodynamics of Metal

Cofactors to *Burkholderia pseudomallei* IspF

Student Name (print or type) _____ Krystal Do _____

Faculty Supervisor (print or type) _____ Dr. James Horn _____

Faculty Approval Signature _____


Department of (print or type) _____ Chemistry and Biochemistry _____

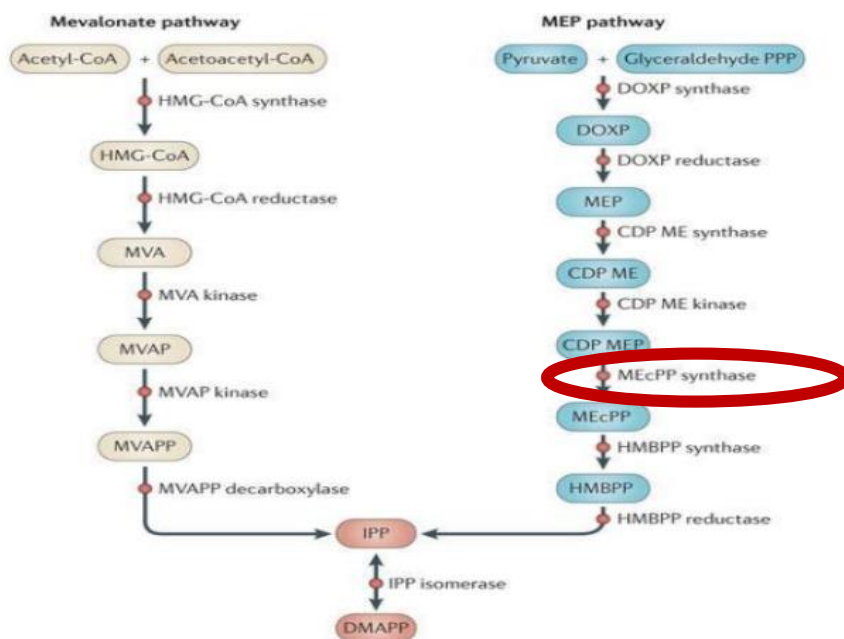
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Abstract

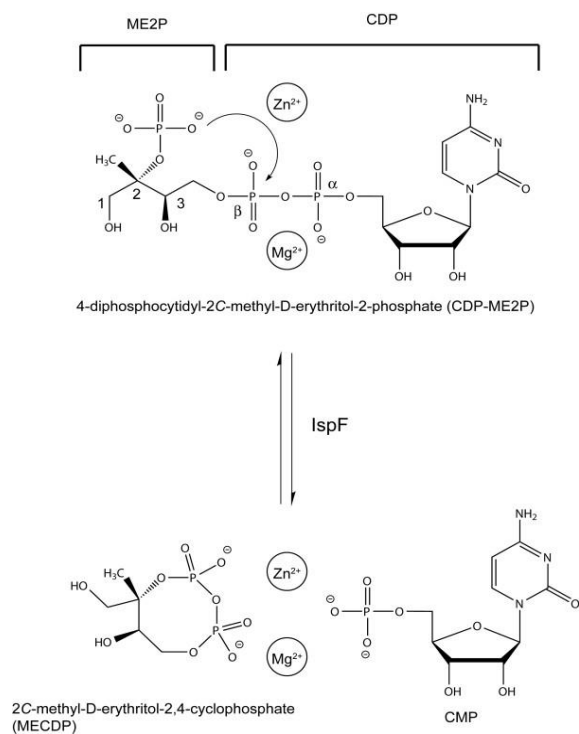
The characterization of the binding properties of the Zinc ion, a metal cofactor, for the IspF enzyme can reveal details of critical interactions that allows to better understand IspF function. The use of Isothermal Titration Calorimetry (ITC) was used to determine the binding affinity of the Zn^{2+} cofactor to the IspF enzyme. A sample of IspF was titrated with a Zinc solution, using a Zinc to Buffer titration as a control. This was done to isolate ligand and protein interactions from the interference of buffer solution. The titration of the Zn^{2+} cofactor can reveal insight into the binding thermodynamics with *Burkholderia pseudomallei* IspF. Further studies will be carried out against another IspF sample from *Escherichia coli*, as well as other 2+ metal ions like Magnesium (Mg^{2+}) and Calcium (Ca^{2+}).

Introduction

Isoprenoids are a group of natural compounds that are vital to cellular functions and membrane stability¹. Two pathways are responsible for the biosynthesis of these compounds are the Mevalonate Pathway (MVA) and the Methylerythritol Phosphate (MEP) Pathway (Scheme 1)². The MVA Pathway is found in eukaryotes, archaea, and some bacteria³. The MEP Pathway is found in bacteria, plants, and some protozoa⁴.



Scheme 1: Flow chart representation of the Mevalonate (MVA) Pathway and the Non-mevalonate (MEP) Pathway



Scheme 2: Reaction mechanism of IspF with metal cofactors Zn^{2+} and Mg^{2+} .³

The MEP Pathway has seven enzymes in the biosynthesis of isoprenoids. IspF is the 5th step in this process, converting CDP-ME2P to MECDP (Scheme 2). The focus of this project is determining the binding affinity of the Zn^{2+} cofactor that is necessary for its catalytic function.

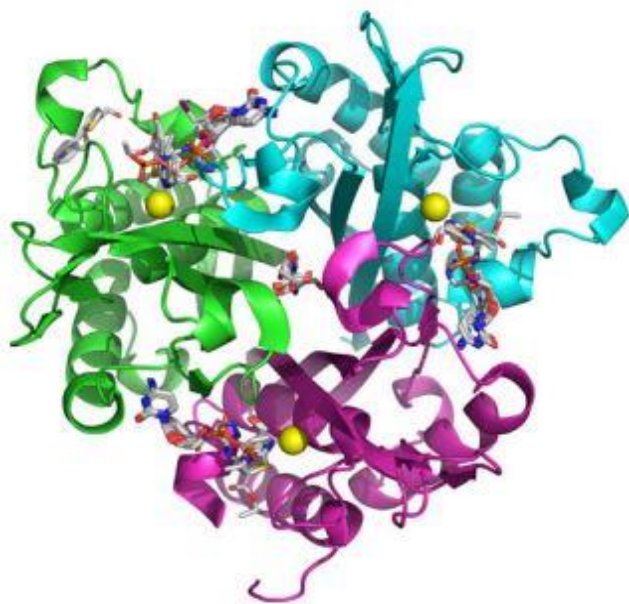


Figure 1: Crystal structure of Burkholderia pseudomallei IspF (PDB: 3P10). Yellow spheres indicate catalytic Zinc ions.

The IspF enzyme binds to the Zinc ion from the organism *Burkholderia pseudomallei*. *Burkholderia pseudomallei* is the bacterium species responsible for melioidosis, also known as Whitmore's disease, and is predominately found within contaminated water and soil of tropical climates⁵. These locations include Northern Australia and Southeast Asia. The bacterium is contacted directly through waterways, ingestion, inhalation, or through cattle or swine. Infection begins in the lungs and can spread to vital organs via bloodstream. Symptoms include fever, headache, and aching of body. Serious infection of the lungs result in large amount of pus and if left untreated can be fatal. In many cases, symptoms do not present or is latent until those infected are at lowest resistance.

Each of the three active sites of the IspF trimer contain a catalytic Zinc ion, which is critical for its catalytic activity. Characterization of the binding properties of the zinc ion for the IspF enzyme will reveal details of a critical interactions that will allow us to better understand IspF function as well as help in the design of new potential IspF inhibitors that target the Zinc ion.

Targeting Zinc in BpIspF can assist in disrupting the MEP Pathway. Non-melvonate pathway (MEP Pathway) is an essential pathway for plant and microbial organism life, not in mammals, specifically humans. By targeting enzymes within the MEP pathway can inhibit microbial growth, which prevents melioidosis from infecting mammals. Resistance is not a concern because *Burkholderia pseudomallei* is completely dependent upon the MEP Pathway to produce isoprenoids. Mammals will not be negatively impacted upon the removal of the catalytic Zinc ion in IspF because they do not depend on the MEP Pathway for existence. Mammals function with their own separate and essential pathway, which is the MVA Pathway (Mevalonate Pathway) to produce isoprenoids. Understanding that the disruption of the MEP Pathway will destroy the bacteria without disrupting the isoprenoid biosynthesis of humans will benefit future drug design through elimination of side effects.

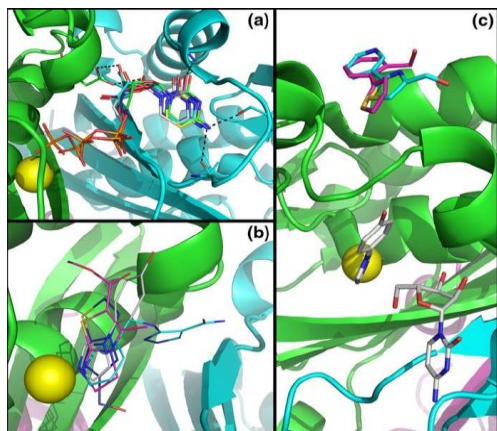


Figure 2: Small molecules which bind BpIspF fall into three distinct categories: a cytidine pocket binders, including cytosine (yellow), cytidine (cyan), 50-iodo-cytidine (green), CMP (navy), CDP (magenta) and CTP (white); b zinc-site binders FOL535 (magenta), FOL717 (navy), FOL8395 (cyan) and FOL955 (white); and c external site binders FOL694 (magenta) and FOL795 (cyan). A single protein crystal structure (PDB ID: 3P10) is, depicted for clarity. Key interactions illustrated in a between cytidine, D48 of one monomer, and A102, P105 and A108 of the opposite monomer (black dashes). Cytidine and FOL955 (white) are illustrated in the active site for c. Figure generated using PyMol.

Methods

Purified BpIspF provided by SSGCID

Organism: *Burkholderia pseudomallei* 1710b

Center Reference ID: BupsA.00122.a

Initial Concentration = 3.77 mM

Final Concentration = 38.23 μ M

Buffer dialysis w/ EDTA multiples times: 10mM EDTA, 200mM NaCl, 25mM Tris, 1% Glycerol, pH 8.0

Zinc is removed from BpIspF using Ethylenediaminetetraacetic acid (EDTA). EDTA⁶ is a chelating agent, which is a chemical that binds strongly to metal ions. The addition of EDTA to the buffer solution will aid in the removal of the catalytic Zinc ion from BpIspF. Once made and set, the enzyme solution is placed inside a semi-permeable membrane in the buffer solution. The solution is set to diffuse overnight to make sure that the Zinc ion diffuses out of BpIspF.

Buffer dialysis w/out EDTA multiple times: 1mM TCEP, 200mM NaCl, 25mM Tris, 1% Glycerol, pH 8.0

Because the presence of EDTA in the enzyme will interfere with the data from ITC, it is mandatory to purify the enzyme without the Zinc ion⁷. A buffer dialysis solution is made without EDTA. Once made and set, the enzyme solution is placed inside a semi-permeable membrane in the buffer solution. The solution is set to diffuse EDTA and remaining Zinc ions attached to EDTA from BpIspF

ZnCl₂ diluted with 2nd dialysis buffer to guarantee buffer matching at a final concentration of 1.005 mM

Approximately 1.005M solution was made with ZnCl₂ was made and diluted further with dialysis buffer (without EDTA).

Binding Thermodynamics determined through Isothermal Titration Calorimetry

MicroCalTM VP-ITC (Isothermal Titration Calorimetry) is used to analyze the binding thermodynamics of BpIspF by adding the zinc ion back into the protein to analyze binding strength. It allows for the determination of binding thermodynamics. A second ITC run is performed between the ligand and buffer to isolate the binding between only the ligand and protein.

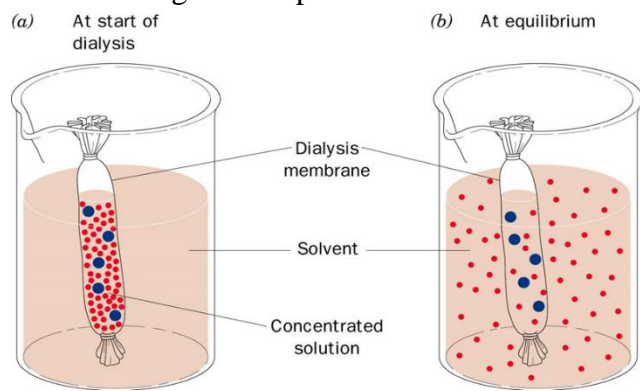


Figure 3: Simple representation of a buffer dialysis

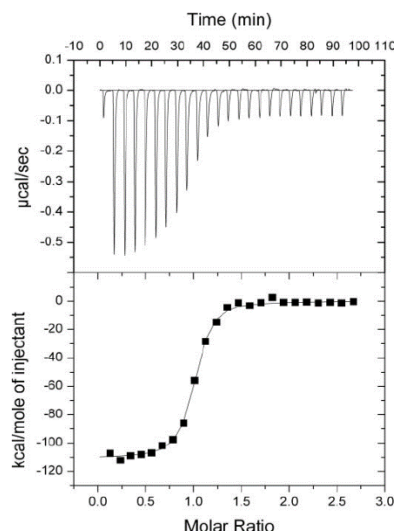


Figure 4: Graphical representation of collected ITC data

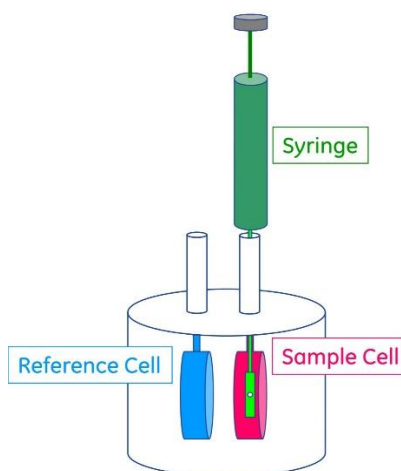


Figure 5: Representation of MicroCal™ VP-ITC instrumentation⁸

Ideally, initial large peaks reveal immediate binding of the Zinc (Zn^{2+}) metal cofactors to the three catalytic binding sites of BpIspF. As binding occurs, there are less sites available for binding to the protein due to saturation of Zinc ions. This, in turn reduces the amount of binding as the titrations of ZnCl_2 progress and creates a sigmoidal binding curve.

Inside the ITC is a reference cell filled with distilled water. The sample cell is filled with the protein, while the syringe contains the ligand (ZnCl_2). The water serves as a comparison cell for heat that occurs as the machine runs. It allows source of the heat generated in the instrument to be known.

Results & Discussion

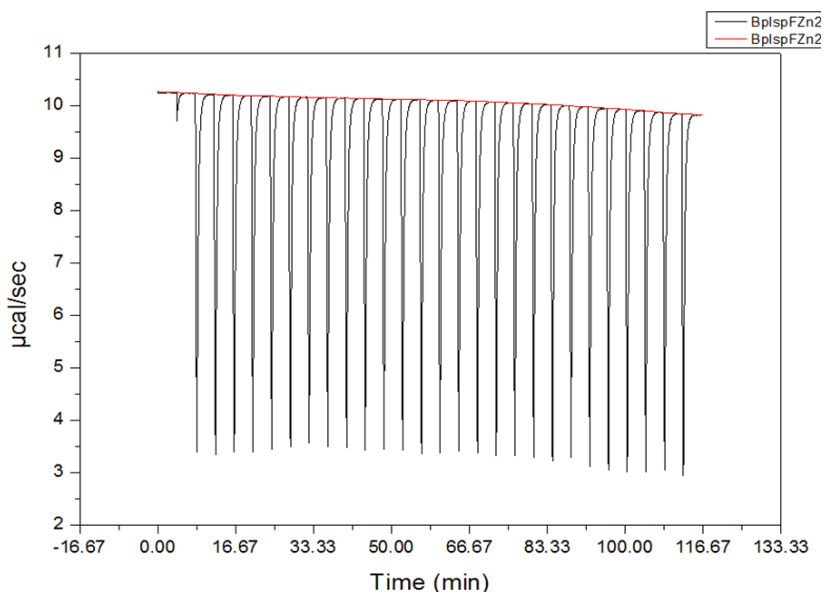


Figure 5: Data retrieved after run of experiment performed

Large peaks are seen during each injection throughout the course of the ITC experimentation. This indicates very strong binding of ligand (Zn^{2+}) to the protein target (BpIspF). No decrease in peak intensity indicating incomplete saturation of binding sites available in the protein sample. The binding curve is represented with a positive linear slope, showing that binding is constantly increasing. Due to this, the data does not fit the appropriate model for ligand binding. Further studies are necessary for actual quantification of the binding affinity of BpIspF to Zn^{2+} .

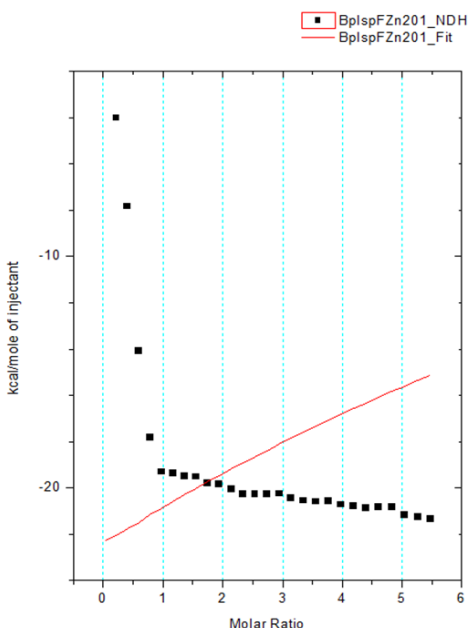


Figure 6: Binding curve of experiment performed

Conclusion

Large peaks reveal immediate binding of the Zinc (Zn^{2+}) metal cofactors to the three catalytic binding sites of BpIspF. In addition, the remnants of EDTA allows for another binding sites for Zinc (due to insufficient multiple buffer dialysis's following Zn^{2+} removal to fully remove EDTA). Once the binding sites are saturated with Zinc, the peaks should decrease in height as these sites are taken up by Zinc metal cofactors as there are less open binding sites for the ligand. Because the concentration of Zinc was not enough to saturate the binding sites of BpIspF, with each titration was immediate binding to the ligand. This is the reason why large peaks continue to appear with titration and do not decrease in height to result in a sigmoidal bind curve.

Further Studies will be conducted using a modified buffer solution and with a greater concentration ratio of the Zinc (Zn^{2+}) metal cofactor to *Burkholderia pseudomallei* IspF in an attempt to further quantify the binding affinity of the Zinc cofactor to BpIspF. Multiple buffer dialysis's will be done after the stripping of the metal cofactor from BpIspF to fully remove EDTA. Studies will also be carried out with other 2+ metal ions, such as Magnesium (Mg^{2+}) which is another ion necessary for catalytic function, and Calcium (Ca^{2+}). The same experimentation will also be carried out against IspF from *Escherichia coli*.

References

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